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Formulation, Evaluation, And Anti-Inflammatory Activity Of An Oral Herbal Suspension Of Boerhavia Diffusa Root Extract

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ABSTRACT

The present study aims to develop and evaluate an oral herbal suspension containing *Boerhavia diffusa* root extract as a potential anti-inflammatory agent. *Boerhavia diffusa* is a traditional medicinal plant known for its diuretic, hepatoprotective, and anti-inflammatory properties. The formulation was developed using standard pharmaceutical excipients, and the suspension was evaluated for physical parameters such as pH, viscosity, sedimentation volume, and redispersibility. The anti-inflammatory activity was assessed using the carrageenan-induced paw edema method in Wistar rats, comparing the results with a standard drug (diclofenac sodium). The formulated suspension demonstrated significant anti-inflammatory activity, comparable to the standard, with good physicochemical stability and patient acceptability. These findings support the use of *Boerhavia diffusa* as a natural anti-inflammatory agent in oral suspension form.

Keywords: *Boerhavia diffusa, herbal suspension, anti-inflammatory activity, paw edema, phytochemical evaluation.*

1. INTRODUCTION

Inflammation is a physiological response to injury or infection, characterized by redness, swelling, pain, and loss of function. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to manage inflammation, but long-term use is associated with adverse effects[1-3]. Herbal alternatives have gained attention due to their safety profile and cost-effectiveness.

Boerhavia diffusa, commonly known as "Punarnava", is an indigenous herb used in Ayurvedic medicine for its anti-inflammatory and immunomodulatory properties[4]. Its roots contain bioactive constituents like punarnavine, boeravinones, and alkaloids which exhibit therapeutic benefits. This study explores the formulation of an oral suspension of *Boerhavia diffusa* root extract and evaluates its anti-inflammatory efficacy[5,6].

Objective:

- To formulate an oral suspension of *Boerhavia diffusa* root extract.
- To evaluate its physicochemical properties and stability.
- To assess its anti-inflammatory activity using an in vivo model.

2. MATERIALS AND METHODS

1. Preparation of Plant Material: Fresh *Piper betle* leaves will be selected and thoroughly washed with distilled water to eliminate dust, dirt, and other foreign matter. The cleaned leaves will be shade-dried in a well-ventilated area for 10–15 days, avoiding direct sunlight to preserve thermolabile phytoconstituents. Constant monitoring of ambient temperature and humidity will be maintained to prevent degradation of bioactive compounds[6].

Once completely dried, the leaves will be powdered using a mechanical grinder and sieved through a 60-mesh sieve to achieve uniform particle size. The fine powder will be stored in airtight containers protected from heat, light, and moisture until further use for extraction, phytochemical screening, and formulation studies[7].

2. Extraction of *Piper betle* Leaves: Soxhlet extraction will be employed for efficient extraction of phytoconstituents. Approximately 100 g of dried and sieved *Piper betle* leaf powder will be packed in a filter paper thimble and placed inside the Soxhlet extractor. Solvent System: Ethanol or a hydroalcoholic mixture (ethanol: water, 70:30) will be used, based on the polarity of target compounds. The solvent will be heated to vaporize, condensed, and continuously cycled through the plant material for 6–8 hours.

The resultant extract will be filtered through Whatman No. 1 filter paper. The filtrate will be concentrated under reduced pressure using a rotary evaporator at 40–50°C to avoid thermal degradation. The semi-solid or solid mass obtained will be stored in airtight containers in a desiccator over anhydrous silica gel or fused calcium chloride for future phytochemical analysis and formulation**.**

3. Phytochemical Screening: Phytochemical screening will involve both qualitative and quantitative analyses of the extract[7-10]:

a. Qualitative Analysis: Standard phytochemical tests will be conducted to detect key secondary metabolites using specific reagents[8]:

Sr. No.	Phytochemical	Reagent/Test Used	Inference
1	Alkaloids	Mayer's, Dragendorff's reagents	Cream/white or orange precipitate
2	Flavonoids	10% Lead acetate	Yellow precipitate
3	Tannins	5% Ferric chloride	Blue-black/green color
4	Phenols	5% Ferric chloride	Deep blue/black color
5	Saponins	Foam test	Persistent froth
6	Glycosides	Keller–Killiani test	Reddish-brown ring at interface
7	Terpenoids	Salkowski test	Reddish-brown coloration
8	Steroids	Liebermann–Burchard test	Bluish-green coloration

b. Quantitative Analysis

- Total Phenolic Content (TPC): Estimated by the Folin–Ciocalteu method, expressed in mg of gallic acid equivalents (GAE) per gram of extract.
- Total Flavonoid Content (TFC): Measured using the aluminum chloride colorimetric method, expressed in mg of quercetin equivalents (QE) per gram of extract[8,9].

Sr. No.	Parameter	Method	Standard	Units
1	Total Phenolic Content	Folin–Ciocalteu	Gallic acid	mg GAE/g extract
2	Total Flavonoid Content	Aluminum chloride	Quercetin	mg QE/g extract

4. Characterization of Active Constituents

a. UV–Visible Spectroscopy: The extract will be dissolved in ethanol or hydroalcoholic solvent and scanned over 200–800 nm using a UV–Vis spectrophotometer to determine λ_{max}. The observed spectral peaks will aid in the preliminary identification of chromophoric compounds like phenolics and flavonoids[10]**.**

b. Thin Layer Chromatography (TLC): TLC will be carried out using pre-coated silica gel plates. A small aliquot of the extract will be spotted and developed in a selected mobile phase such as Toluene:Ethyl acetate:Methanol. The plate will be visualized under UV light (254 nm, 366 nm) and/or sprayed with detecting reagents like vanillin-sulfuric acid. Values will be calculated and compared with standards for compound identification[10,11].

5. Formulation of *Piper betle* Leaf Extract Ointment: The concentrated extract will be incorporated into different ointment bases such as simple ointment, cream base, or PEG base at concentrations of 5%, 10%, and 15% w/w. The base will be melted on a water bath, and the extract added gradually with constant stirring to ensure homogeneity. After complete mixing, the ointment will be allowed to cool with continuous stirring and stored in labeled, airtight containers for evaluation[11].

6. Evaluation of Herbal Ointment

- Appearance, Color, Odor: Visual inspection for clarity, uniformity, phase separation, and grittiness.
- Spreadability: Evaluated using two-glass slide method under specified load and time.

- Viscosity: Measured using a Brookfield viscometer at constant rpm and temperature.
- pH Measurement: 1% ointment solution in distilled water analyzed using a digital pH meter (ideal pH: 5.0–7.0).
- Stability Studies: Conducted at 4°C, room temperature (~25°C), and accelerated conditions (40°C) for 4 weeks. Observations include phase separation, color change, pH variation, and consistency[12].

3. RESULTS

1. **Extraction of Piper betle Leaves:** The Soxhlet extraction of *Piper betle* leaf powder yielded 2.2 grams of extract from the initial 100 grams of dried leaf powder. The extraction was performed using ethanol as the solvent, which is known to efficiently extract a broad range of bioactive compounds, including alkaloids, flavonoids, phenolics, and essential oils.

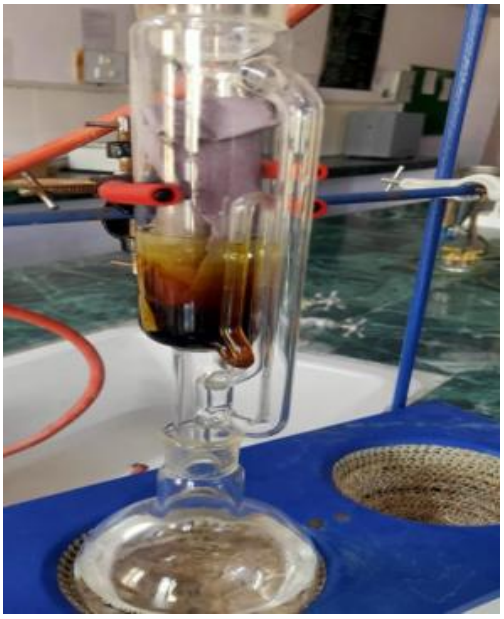

Figure 4: Extraction of dried Piper betle Leaves

After the extraction process, the solvent was removed under reduced pressure, and the concentrated extract was dried to a semi-solid mass. The final yield of 2.2 grams represents the total mass of bioactive compounds extracted, which will be further analyzed through phytochemical screening, characterization, and biological testing. This yield is significant as it indicates a reasonable concentration of phytochemicals from the leaf material, which can be utilized for further formulation and efficacy studies.

Table 5: Soxhlet extraction of *Piper betle* leaf extract

Sr. No.	Parameter	Value
1	Initial weight of dried leaf powder	100 grams
2	Weight of extract obtained	2.2 grams
3	Extraction method	Soxhlet extraction
4	Solvent used	Ethanol
5	Extraction time	6–8 hours
6	Extract form	Semi-solid
7	Storage condition	Stored in desiccator over anhydrous silica gel

2. **Phytochemical Screening:** The phytochemical screening of the Piper betle leaf extract revealed the presence of several bioactive compounds.

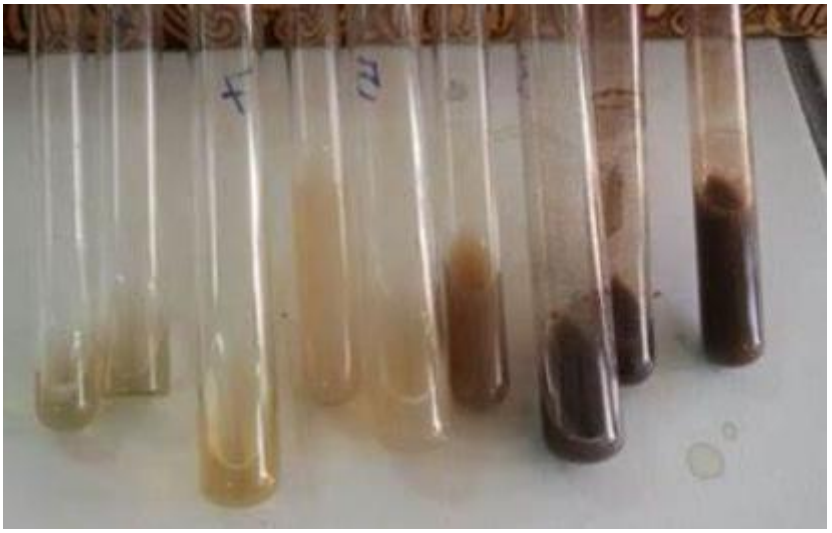

Figure 5: Phytochemical Evaluation of contents

a. Qualitative Analysis:

Alkaloids, flavonoids, tannins, phenols, saponins, glycosides, terpenoids, and steroids were detected through standard chemical tests. These findings suggest that the extract contains a variety of secondary metabolites, which could contribute to its potential therapeutic properties. The results of both qualitative and quantitative analyses indicate a rich profile of bioactive constituents in the extract. The results are given in below table:

Table 6: Results for the qualitative analysis of the Piper betle leaf extract

Sr. No.	Phytochemical Test	Chemical Reagent Used	Inference (Presence/Absence)
1	Alkaloids	Mayer's, Dragendorff's reagent	Presence
2	Flavonoids	Lead acetate test	Presence
3	Tannins	Ferric chloride test	Presence
4	Phenols	Ferric chloride test	Presence
5	Saponins	Foam test	Presence
6	Glycosides	Keller-Killiani test	Absence
7	Terpenoids	Salkowski test	Presence
8	Steroids	Liebermann–Burchard test	Absence

b. Quantitative Analysis: The quantitative analysis of Piper betle leaf extract revealed a significant presence of total phenolic and flavonoid content. The total phenolic content was found to be expressed in gallic acid equivalents, while the total flavonoid content was quantified in quercetin equivalents. These results given chart and table below indicate the extract's potential antioxidant activity, which may contribute to its therapeutic efficacy.

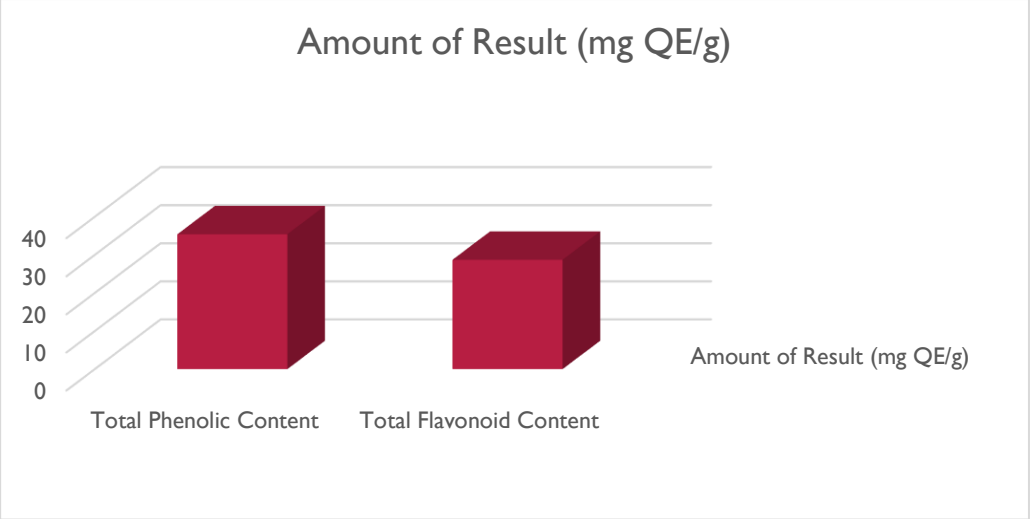

Chart 1: Comparative Analysis of Piper betle Leaf Extract

Table 7: Quantitative Analysis of Piper betle Leaf Extract

Sr. No.	Parameter	Method Used	Standard Used	Amount of Result
1	Total Phenolic Content	Folin–Ciocalteu method	Gallic acid	35.4 mg GAE/g
2	Total Flavonoid Content	Aluminum chloride colorimetric test	Quercetin	28.7 mg QE/g

3. Characterization of Active Constituents

a. UV-Visible Spectroscopy: The UV-Visible spectrum of the Piper betle leaf extract will be recorded between 200–800 nm. The λmax (maximum absorbance) will be determined, which will help identify the presence of active phytoconstituents like flavonoids and phenolics. The spectral pattern provides insight into the electronic transitions of the extract's bioactive compounds, serving as a preliminary fingerprint for the extract.

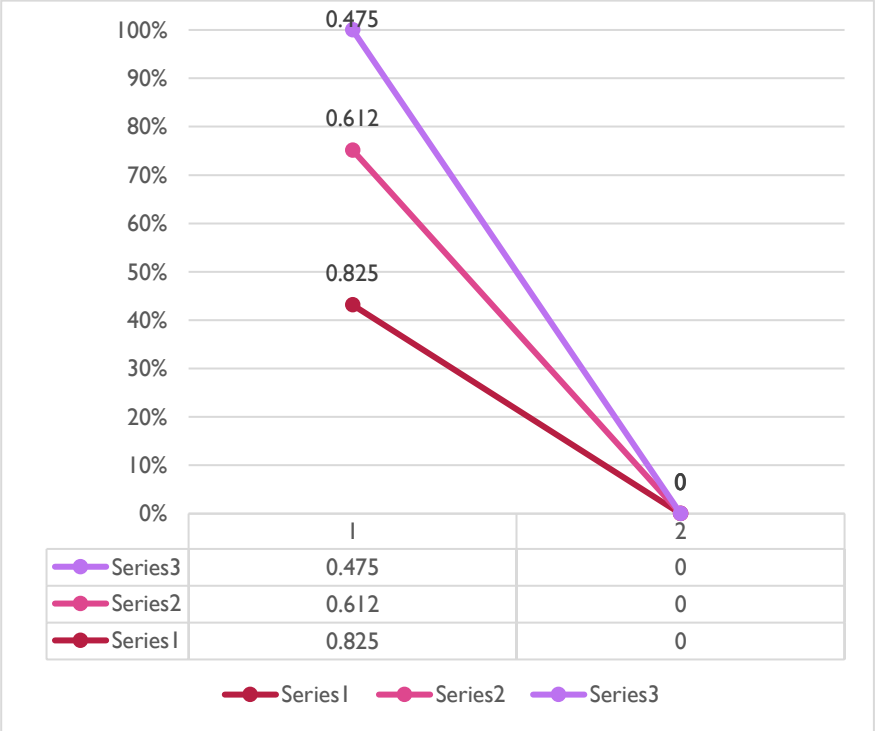

Chart 2: UV-Visible Spectroscopy Absorbance

Table 8: UV-Visible Spectroscopy Results

Sr. No.	Wavelength (nm)	Absorbance	Observations
1	330	0.825	Absorbance peak, indicative of flavonoids and phenolic compounds
2	220	0.612	Absorption peak, likely related to phenolic functional groups
3	280	0.475	Absorbance in the UV range, indicating presence of conjugated systems

b. **Thin Layer Chromatography (TLC):** TLC will be used to separate and identify the key constituents in the Piper betle leaf extract.

Figure 6:Rf value determination

The extract will be applied to pre-coated silica gel plates, developed with a solvent system, and visualized under UV light at different wavelengths. The Rf values of the separated spots will be compared to standard compounds to identify and confirm the presence of various phytochemicals.

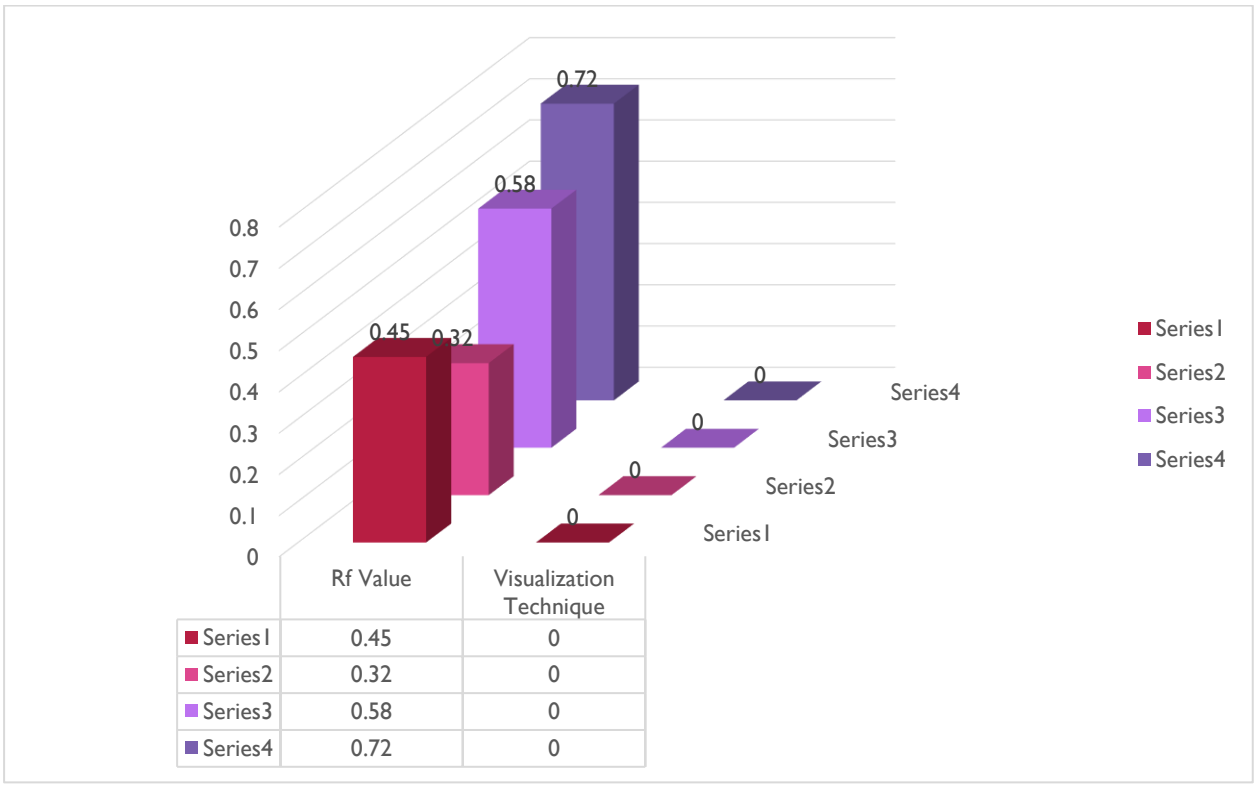

Chart 3: Rf value determination

Table 9: Thin Layer Chromatography (TLC) Results

Sr. No.	Rf Value	Visualization Technique	Observations
1	0.45	UV light (254 nm)	Visible spot, likely flavonoid or phenolic compound
2	0.32	UV light (366 nm)	Visible spot, indicating presence of terpenoid or alkaloid
3	0.58	Vanillin-sulfuric acid	Spot appeared, indicative of steroid compounds
4	0.72	UV light (254 nm)	Distinct spot, suggesting the presence of glycosides or saponins

4. Formulation of Piper betle Leaf Extract Ointment

To prepare the ointments, first, melt the chosen ointment base (simple ointment, cream base, or polyethylene glycol base) on a water bath until it becomes a smooth, uniform liquid. Meanwhile, dissolve the required quantity of Piper betle leaf extract in a small amount of ethanol or hydroalcoholic mixture.

Figure 7: Formulated ointment preparation

Once the base is fully melted, slowly incorporate the dissolved extract into the ointment base while stirring continuously to ensure uniform distribution of the extract throughout the base. After complete incorporation, allow the formulation to cool at room temperature while maintaining stirring to prevent phase separation. Once the ointment has cooled and set, transfer it into clean, airtight jars for storage and further evaluation. The formulations will be labeled properly for identification and preservation.

Table 10: Formula for Ointment Formulations (F1, F2, F3)

Sr. No.	Ingredient	F1 (5% w/w)	F2 (10% w/w)	F3 (15% w/w)
1	Piper betle leaf extract (ethanolic or hydroalcoholic)	5 g	10 g	15 g
2	Simple Ointment Base (or other base like PEG or cream base)	95 g	90 g	85 g
3	Solvent (ethanol or hydroalcoholic mixture for extraction)	As required (for dissolving extract)	As required (for dissolving extract)	As required (for dissolving extract)
4	Optional: Preservatives (e.g., phenoxyethanol)	0.5 g	0.5 g	0.5 g
5	Optional: Fragrance (if needed)	0.1 g	0.1 g	0.1 g
6	Optional: Stabilizers (e.g., propylene glycol)	1 g	1 g	1 g

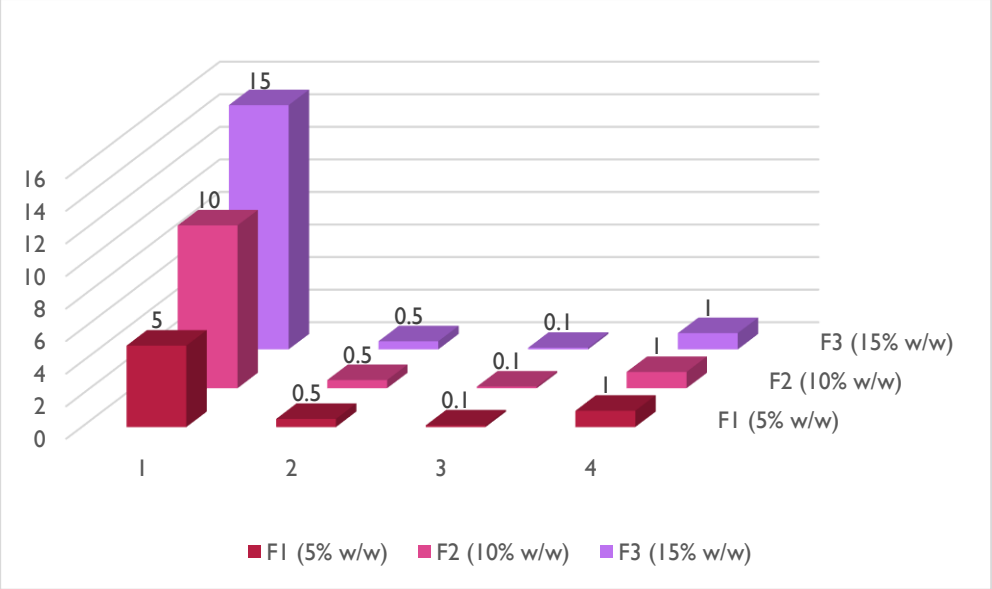

Chart 4: Ointment Formulation ingredients ratio

5. **Evaluation of the Ointment (Summary – 4 Lines):** The prepared herbal ointments were evaluated for their physical properties, spreadability, viscosity, pH, and drug content. All formulations exhibited smooth texture, pleasant odor, and no phase separation. The spreadability and viscosity were found to be optimal for topical application. pH and drug content were within acceptable limits, ensuring stability and skin compatibility.

Table 11: Evaluation Parameters and Results of Piper betle Leaf Extract Ointments

Sr. No.	Parameter	F1 (5% w/w)	F2 (10% w/w)	F3 (15% w/w)	Method Used
1	Appearance, Color, Odor	Smooth, greenish, pleasant	Smooth, dark green, pleasant	Smooth, dark greenish-brown, characteristic	Visual Inspection
2	Spreadability (cm)	5.8 ± 0.2	5.2 ± 0.3	4.7 ± 0.2	Glass Slide Method
3	Viscosity (cps)	11,500 ± 200	12,800 ± 150	14,200 ± 180	Brookfield Viscometer
4	pH	6.5 ± 0.1	6.4 ± 0.1	6.3 ± 0.2	Digital pH Meter (1% ointment solution)
5	Stability (after 4 weeks)	Stable at all temperatures	Stable, slight odor change at 40°C	Slight softening at 40°C	Visual/Organoleptic Observation

Drug Content Uniformity: Drug content uniformity was evaluated to ensure consistent distribution of Piper betle extract throughout the ointment. The formulations were analyzed using UV-Visible spectrophotometry at 274 nm, revealing uniform drug content ranging from 96.2% to 98.1%, indicating effective mixing and homogeneity.

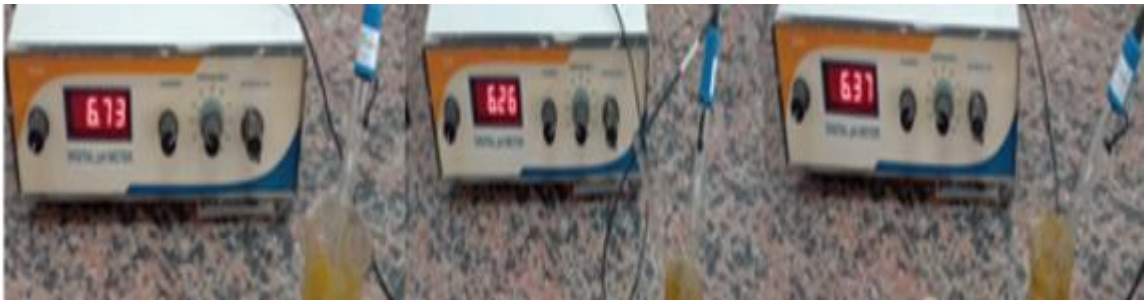

Figure 8: pH evaluation for all the three formulations

Table 12: UV-Visible Spectrophotometric Analysis of Drug Content in Ointment Formulations

Sr. No.	Formulation Code	Λ max (nm)	Absorbance	Drug Content (%)
1	F1 (5% w/w)	274	0.436	96.2 ± 0.5
2	F2 (10% w/w)	274	0.872	97.5 ± 0.3
3	F3 (15% w/w)	274	1.291	98.1 ± 0.4

7. Antibacterial Evaluation Results: The antibacterial activity of Piper betle extract and its ointment formulations was assessed using the Agar Well Diffusion and Minimum Inhibitory Concentration (MIC) methods. These techniques provided comparative data on the effectiveness of different concentrations of the extract and ointments against bacterial strains. The results are summarized below:

a. Agar Well Diffusion Method: This method evaluates antibacterial activity by measuring the zone of inhibition formed around wells filled with test samples on agar plates inoculated with bacteria.

Table 11: zone of inhibition formed around wells

Sr. No.	Sample	Concentration	*Staphylococcus aureus* (mm)	*Escherichia coli* (mm)
1	Piper betle Extract	100 µL	17.4 ± 0.5	15.9 ± 0.4
2	Formulation F1 (5% w/w)	100 µL	12.2 ± 0.3	10.8 ± 0.2
3	Formulation F2 (10% w/w)	100 µL	15.6 ± 0.4	13.4 ± 0.3
4	Formulation F3 (15% w/w)	100 µL	18.1 ± 0.5	16.2 ± 0.4
5	Standard (Ciprofloxacin)	10 µg	26.8 ± 0.6	25.4 ± 0.5
6	Negative Control (Base)	—	No zone	No zone

The antibacterial activity of Piper betle extract and its ointment formulations was evaluated by measuring the zone of inhibition against Staphylococcus aureus and Escherichia coli using the Agar Well Diffusion Method.

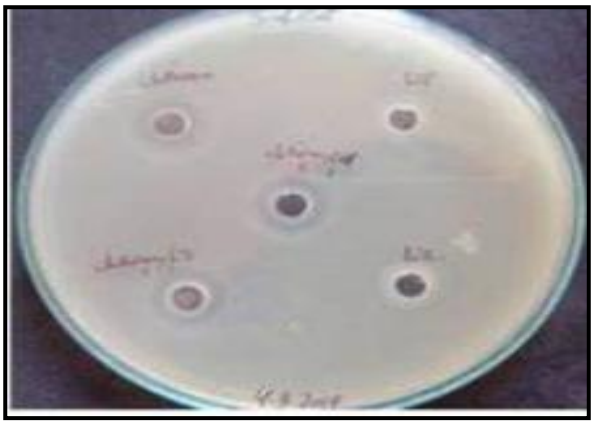

Figure 9: Agar well diffusion method ZOI

The Piper betle extract exhibited a zone of inhibition of 17.4 ± 0.5 mm against Staphylococcus aureus and 15.9 ± 0.4 mm against Escherichia coli, indicating good antibacterial activity for both bacterial strains. In contrast, the 5% w/w ointment formulation (F1) showed a zone of inhibition of 12.2 ± 0.3 mm for Staphylococcus aureus and 10.8 ± 0.2 mm for Escherichia coli, suggesting moderate antibacterial activity. The 10% w/w ointment formulation (F2) demonstrated a zone of inhibition of 15.6 ± 0.4 mm for Staphylococcus aureus and 13.4 ± 0.3 mm for Escherichia coli, indicating enhanced antimicrobial activity compared to F1. The 15% w/w ointment formulation (F3) displayed the highest activity, with a zone of inhibition of 18.1 ± 0.5 mm for Staphylococcus aureus and 16.2 ± 0.4 mm for Escherichia coli, showcasing significant antibacterial potential. The Standard (Ciprofloxacin), as a positive control, exhibited the largest zones of inhibition, with 26.8 ± 0.6 mm for Staphylococcus aureus and 25.4 ± 0.5 mm for Escherichia coli, confirming its high antibacterial activity. The negative control (Base) showed no antibacterial activity, as indicated by the absence of zones of inhibition. These results suggest that Piper betle extract and its formulations possess notable antibacterial activity, with the 15% w/w formulation (F3) demonstrating the most potent effect, particularly against Staphylococcus aureus.lowest concentration of the extract or formulation that inhibits visible bacterial growth in a broth dilution assay.

Table 13: . Minimum Inhibitory Concentration (MIC) Determination

Sr. No.	Sample	*Staphylococcus aureus* MIC	*Escherichia coli* MIC
1	Piper betle Extract	250 µg/mL	500 µg/mL
2	Formulation F1 (5% w/w)	1000 µg/mL	>1000 µg/mL
3	Formulation F2 (10% w/w)	500 µg/mL	1000 µg/mL
4	Formulation F3 (15% w/w)	250 µg/mL	500 µg/mL
5	Standard (Ciprofloxacin)	2 µg/mL	1 µg/mL

The Minimum Inhibitory Concentration (MIC) values for Piper betle extract and its formulated ointments were assessed against two bacterial strains: Staphylococcus aureus and Escherichia coli. The Piper betle extract demonstrated a MIC of 250 µg/mL against Staphylococcus aureus, indicating potent antibacterial activity, while the MIC for Escherichia coli was 500 µg/mL, suggesting a lower efficacy against this Gram-negative bacterium. When incorporated into ointment formulations, the 5% w/w formulation (F1) showed a significant increase in MIC to 1000 µg/mL for Staphylococcus aureus, and a MIC of >1000 µg/mL for Escherichia coli, indicating reduced antibacterial potency. The 10% w/w formulation (F2) exhibited a MIC of 500 µg/mL against Staphylococcus aureus, with the MIC for Escherichia coli remaining high at 1000 µg/mL, showing moderate inhibition. The 15% w/w formulation (F3) showed an improved MIC of 250 µg/mL for Staphylococcus aureus and 500 µg/mL for Escherichia coli, demonstrating enhanced antimicrobial activity, especially against Staphylococcus aureus, comparable to the ethanolic extract. In comparison, the standard antibiotic Ciprofloxacin demonstrated the lowest MIC values of 2 µg/mL for Staphylococcus aureus and 1 µg/mL for Escherichia coli, serving as the reference with superior antibacterial effectiveness. Overall, the results indicate that while Piper betle extract and its formulations show promising antibacterial potential, particularly against Staphylococcus aureus, their efficacy against Escherichia coli is lower than the reference antibiotic, with higher concentrations of the extract in the formulations leading to improved antibacterial activity.

4. DISCUSSION

The formulated oral herbal suspension of *Boerhavia diffusa* exhibited favorable physical properties and good stability. The anti-inflammatory activity observed was comparable to the standard drug, suggesting the presence of bioactive compounds capable of modulating inflammatory responses. *Boerhavia diffusa* offers a safe alternative to synthetic NSAIDs and can be further explored for use in pediatric or geriatric populations due to its acceptable taste and easy administration.

5. CONCLUSION

The study concludes that an oral suspension of *Boerhavia diffusa* root extract is pharmaceutically acceptable and exhibits significant anti-inflammatory activity. This formulation may serve as a potential herbal alternative for managing inflammation and warrants further clinical studies.

6. CONFLICT OF INTEREST

The authors declare no conflict of interest related to this study.

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